PCT/EP 99-07911

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Patentanmeldung Nr. | Patent application No. | Demande de brevet n°

98203501.6

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Page 2 de l'attestation

Anmeldung Nr.:
Application no.:
Demande n°: 98203501.6

Anmeldetag:
Date of filing:
Date de dépôt: 20/10/98

Anmelder:
Applicant(s):
Demandeur(s):
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Bezeichnung der Erfindung:
Title of the invention:
Titre de l'invention:
Enteral composition

In Anspruch genommene Priorität(en) / Priority(ies) claimed / Priorité(s) revendiquée(s)

Staat: State: Pays:	Tag: Date: Date:	Aktenzeichen: File no. Numéro de dépôt:

Internationale Patentklassifikation:
International Patent classification:
Classification internationale des brevets:
A23L1/305, A23J1/20, A61K38/20

Am Anmeldetag benannte Vertragstaaten:
Contracting states designated at date of filing: AT/BE/CH/CY/DE/DK/ES/FI/FR/GB/GR/IE/IT/LI/LU/MC/NL/PT/SE
Etats contractants désignés lors du dépôt:

Bemerkungen:
Remarks:
Remarques:

Enteral composition

The present invention relates to a new enteral composition, for example an infant formula, processes to manufacture this formula, and the use of this formula to feed mammals in need thereof.

State of the art

Infant formula are generally designed to resemble human milk as closely as possible. However, many constituents in human milk are bioactive and, because of synergies among these components, the inclusion of just one or a few of these components may not reproduce in the infant formula the bioactivity observed in the human milk.

In addition bioactivity of these components may be affected by heat treatment for sterilization and long term storage of the formula.

Also not all of these constituents have been identified. Therefore there are difficulties in formulating infant formulad to resemble human milk.

CD14 is a myeloid cell-surface glycoprotein, which acts as the main receptor for bacterial lipopolysaccharide. It is well documented that monocyte/macrophage activation by lipopolysaccharides via membrane CD14 (mCD14) triggers the release of a variety of pro-inflammatory, immunoregulatory and cytotoxic molecules , such as TNF-α, IL-1, IL-6, IL-8, oxygen radical products and nitric oxide. Importantly, mCD14 lacks transmembrane and cytoplasmic domains , and it is anchored to the cell membrane by a glycosyl-phosphatidylinositol linkage.

In addition to the membrane bound form, soluble CD14 (sCD14) has been identified in normal serum. Serum sCD14 exists in two forms, sCD14α (49 kDa) and sCD14β (55 kDa). It has been demostrated that sCD14 binds lipopolysaccharides and mediates the lipopolysaccharide–induced activation of cells that lack mCD14, including epithelial and endothelial cells and astrocytes, as well as mCD14 expressing cells, such as monocytes and neutrophils . Recently, the first biochemical characteristics of cell surface structures that appear to serve as the receptor for complexes of sCD14 and lipopolysaccharides have been identified (Frey E.D. et *al.* J. Exp. Med. 1992, 176: 1665-1671; Labeta MO *et al.*

Eur J Immunol 1993, 23:2144-2151; Durieux *et al.* Eur. J. Immunol 1994, 24:2006-2012; Vita N. *et al.* J. Immunol 1997, 158:3457-3462).

The main source of sCD14 in normal plasma is the monocyte. Monocytes release the two isoforms of sCD14, α and β. The former is produced by limited proteolysis from membrane-bound CD14 and the latter is directly derived from the intracellular compartment (Durieux *et al.*, 1994). The two soluble forms of CD14 are found in human plasma. The sCD14β:sCD14α ratio in culture supernatant of normal monocytes is approximately 2:1. However, in plasma from normal donors the sCD14α levels are either similar to, or even higher than, those of sCD14β, suggesting that the amount of sCD14β released *in vivo* is lower or that other cell types may contribute to the plasma pool.

A substantial concentration of sCD14 is found in normal human plasma, 2-3 μg/ml. In sera of septic patients global concentrations of sCD14 are elevated, reaching around 4 μg/ml. It has been reported a correlation between high levels of sCD14 at the onset of septic shock and poor outcome in septic patients (Landmann *et al.* J. of Inf. Diseases, 1995, 171: 639-644).

In addition it has been demonstrated sCD14 has a protective effect against bacterial challenge when it is used at relatively high concentrations (Bazil V. *et al.* J. Immunol. 1991,147:1567-1574; Schütt C. *et al.* Res Immunol 1992, 143: 71-78; Haziot A. *et al* J. Immunol. 1994, 152: 5868-5876).

Although it is clear that sCD14 plays a role in the ummune system, the presence of sCD14 in human milk has not been investigated.

It is an object of this invention to provide an infant formula which resembles, as much as possible, breast milk.

Summary of the invention

According, this invention provides an enteral composition including an effective amount of soluble CD14.

A second aspect of the invention relates to the use of sCD14 for the manufacture of a dietary composition for the treatment or prevention of diseases of the gastro-intestinal tract of mammals in need thereof, or for contributing to the normal intestinal development during neonatal colonisation.

In a third aspect, the invention provides a process to manufacture an enteral composition comprising the step of adding sCD14 to the composition.

The enteral composition may be an infant formula, clinical nutrition formala or animal feed.

Brief description of the drawings

Figure 1A illustrates a comparative SDS-PAGE pattern of sCD14 in human breast milk (several dilutions (from 1:6 to 1:100) and in normal human plasma serum (NP, 1:50), with a rabbit polyclonal antibody.

Figure 1B illustrates the lack of detection of the peptide of sCD14 in a powdered bovine milk.

Detailed description of the invention

This invention is based upon the discovery that human milk contains sCD14. In particular, a sCD14α-like form (approximately 48 kDa) is been found to be the main species of sCD14 of mother's milk of more than 7 days after delivery. By incorporating CD14, and in particular the sCD14α form into an infant formula, the infant formula may more closely resemble human milk.

In this specification, the terms AMP, CMP, GMP and UMP mean respectively the monophosphates of adenosine, cytidine, guanosine and uridine and their nucleotide equivalents which include polymeric RNA, ribo-nucleosides, ribonucleoside containing adducts and di-and triphosphate ribo-nucleotides.

The enteral composition of the invention comprises an effective amount of sCD14, and preferably contains at least about 25 μg/ml of sCD14. The other

components of the enteral composition are those normally encountered, and may be those described below.

The sCD14 may be any form of sCD14, but is preferably the sCD14α form. More preferably, the sCD14 is the form found in human milk and recognised by a polyclonal rabbit antibody. This form has a molecular mass of approximately 48 KDa, an electrophoretic mobility faster than the serum alpha form and cannot be recognised by an antibody directed against the 8 aa at the C terminal of the serum beta form. The CD14 may be extracted from bovine, buffalo, goat or sheep milk. Alternatively it can be produced by recombinant microorganisms; for example recombinant fungi or yeast.

In view of the high levels in this natural source and the proven biological activity, the supplementaion of baby formula with this soluble mediator is expected to be of physiological benefit during the neonatal period characterised by bacterial challenge due to bacterial colonisation and antigen challenge that could lead to allergic reactions.

The invention is also directed to the use of sCD14 for the manufacture of a dietary composition for the treatment or prevention of disorders of the gastro-intestinal tract of mammals in need thereof. In particular these disorders include necrotising enterocolitis, neonotal sepsis, infectious diarrhoea, disbalance of the intestinal microflora, allergic reactions to food, bacterial translocation from the gut to other compartments of the body, etc. Mammals that are likely to develop these disorders are premature, mal-nourished, immuno-depressed, and mammals under trauma conditions.

The enteral composition may contain about 1.8-4.5 g protein/100 kcal, preferably about 1.8-3.6 g/100 kcal. The protein may be any suitable protein such as cow's milk proteins, casein, whey, soy protein, egg protein, pea protein and mixture thereof. The protein may be in the form of salts, such as caseinates, isolates and concentrates. Further the protein may be in intact or hydrolysed form. If desired, free amino acids may also be used. Preferably a mixture of the whey : casein mass ratio is 60:40.

The whey can be prepared to have reduced allergenicity using conventional techniques such as described in U.S. Patent 5039532. For example, it can be prepared by electrodialysis or ultrafiltration.

The enteral composition may contain about 7 g to 14 g/100 kcal of carbohydrate, provide 40-60 % of calories as carbohydrate. The carbohydrate can be supplied in any conventional form including in simple form and complex form. Simple carbohydrates include lactose, maltose, sucrose and corn syrup solids. Complex carbohydrates include starches and maltodextrins. The starch may be precooked or pregelitanised.

The enteral composition may contain about 3.3 to 6.5 g/100 kcal of fat. The fat can be supplied in any suitable form including saturated fats, monounsurated fats (MUFA), polyunsaturated fats (PUFA) or a mixture thereof. Preferably the fat is provided as 1/3 saturated fat, 1/3 MUFA and 1/3 PUFA. Saturated fats include butyric, valeric, caproic, capylic, decanoic, lauric, myristic, palmitic, steraic, arachidic, behenic and lignoceric. MUFAs include palmitoleic, oleic, elaidic, vaccenic and erucic. Preferred PUFAs are C18, C20 or C22 ω-3 or C18, C20 or C22 ω-6 ployunsaturated fatty acids. Preffered are the C20 or the C22 ω-3 or C20 or C22ω-6 polyunsaturatted fatty acids.

These include not only arachidonic acid (ARA) but also eicosapentaenoic acid (EPA) and docoshexaenoic acid (DHA).

More than one PUFA can be added. In this case, two or more PUFAs may be from a different source, and therefore one may add either the PUFAs separately (as separate compositions) or mix the two PUFAs (to give a single compositions) before addition during the foodstuff preparation process. For example, fish oil contains DHA which may be mixed with one or more microbial oils containing another PUFA (e.g. ARA).

The enteral composition may have a carbohydrate : lipid weight ratio which is greater than 60:40. Preferably the ratio of carbohydrate to lipid is between 65:35 and 90:10. More preferably still it is between 70:30 and 85:15.

The enteral composition of the present invention can additionally contain nutritionally acceptable quantities of the following minerals and vitamins: calcium, phosphorus, potassium, sodium, chloride, magnesium, iron, copper, zinc, manganese, iodine, selenium, vitamin A, vitamin D, vitamin E, vitamin K1, vitamin B1, vitamin B2, vitamin B6, vitamin B12, vitamin C, pantothenic acid, niacin, folic acid, biotin, choline, and inositol. The composition can also contain other nutritional factors including taurine, carnitine, etc. The enteral composition may contain at least 7.5 mmoles of carnitine /100 kcal.

The enteral composition may contain one or more of AMP, CMP, GMP and UMP.

The enteral composition of the present invention can additionally include pharmaceutically acceptable fillers, color additives, taste modifiers, non-antagonistic antibiotics, pharmaceuticals and medicaments.

The enteral composition of the present invention can be in a form suitable for ingestion orally or for administration by enteral tube feeding. Oral formulations are however preferred.

The enteral composition of the present invention is suitably formulated to provide 600-800 kcals/litre, preferably 670 kcals/litre. The total volume per dose is 50-200 ml preferably 100-150 ml.

The enteral composition can be a solid, in which case it is preferably dried, and optimally in the form of a powder. Preferably, it is miscible or dispersible in an aqueous liquid, such as water.

The enteral composition can also be in a liquid form, which is ready for use, or a concentrated liquid form which can be diluted before use with water.

The enteral composition of the present invention is usually prepared by a general process comprising the following steps; (1) Standardising pasteurised milk (skimmed, evaporated or whole milk) by the addition of whey protein concentrate, minerals, water-soluble vitamins, trace elements and carbohydrates at high temperatures, for example 60°C, (2) mixing vegetable oil, oil-soluble emulsifiers,

oil-soluble vitamins and antioxidants at high temperatures, for example 60°C, (3) adding the oil mixture obtained from (2) to the standardised milk obtained from (1) with sufficient agitation to allow mixing, (4) homogenising the mixture obtained in (3) in two stages at high temperature and pressure, for example 60°C at 150 and 30 bar, (5) cooling the emulsion obtained under (4) to a low temperature, for example 5°C, (6) adding water-soluble vitamins, minerals and trace elements to the cooled emulsion if desired, (7) sterilising the emulsion obtained under (6) on-line at ultra high temperature (UHT) and/or in appropriate containers to obtain a formula in the form of a sterile liquid or pasteurising and spray drying the emulsion (6) to give a spray dried powder which is filled into appropriate containers and (8), if desired, adding other dry ingredients, e.g. vitamins, minerals, trace elements, whey protein concentrate and carbohydrates to the spray dried powder by dry mixing.

The enteral composition may be supplemented with CD14 by adding the CD14 to the oil during processing. The reason for this is the production plant, or the process need not be significantly modified.

Adding the CD14 at such an early stage can however have disadvantages because the CD14 becomes degraded. CD14 is added preferably at a later stage. This can then minimise the exposure of the CD14 to unfavourable conditions. Preferably, the CD14 is added after drying.

The CD14 can be added in a variety of forms. It may be added as part or component of a liquid or solid composition. If liquid, this may be a lipid composition and /or an oil. The oil may contain solely the CD14 or it may contain a number of other ingredients. If a solid composition is used, the CD14 may be encapsulated in capsules or it maybe in a powdered form, for example.

In the process of the invention it is preferred that the starting oil phase does not contain any PUFAs. This is because they are suitably added later, preferably after drying.

The enteral composition and the use of this enteral composition according to the invention are described in more detail in the biological tests and the examples below where the percentages are given by weight, except when otherwise indicated.

Test 1: Ion exchange chromatography for purification of sCD14 from milk (bovine, buffalo, goat or sheep) and supernantant of CD14 cDNA transfected cells.

The purification of sCD14 from milk or culture supernantant of transfectant cells are performed by ion exchange chromatography. Diluted milk samples or conditioned medium of CD14 cDNA transfectant cells are applied to a Mono Q10/10 column equilibrated with 20mM ethanolamine pH 9.5 . After washing, the column is subjected to a linear gradient of NaCl (0-500mM) in the equilibrating buffer. Fractions are collected and their sCD14 content is determined by a soluble CD14 ELISA.

Further characterization by reverse phase HPLC is carried out to confirm the purity of the protein. The selected fractions are pooled , desalted with PBS, and kept aliquoted at – 70°C until further use . The purified material is routinely characterised by N-terminal sequencing , amino acid analysis, SDS-Page, and mass spectrometry.

Test 2: Detections of sCD14 forms in milk.

Western-Blot assays were carried out so as to detect the sCD14 forms in human milk and bovine milk preparations.

For the human milk, 14 lactating mothers agreed to donate breast-milk samples in quantities (10-60ml) that did not jeopardise the baby nutrition. The human milk samples were obtained at different times after delivery (0-70 days) with a sterile pump. For the bovine milk, a commercial powder milk (pre-BeBa®, Suisse), and a bovine colostrum were also used.

Rabbit polyclonal anti-CD14 was used for the detection of sCD14 in breast milk. This polyclonal antibody was prepared from human recombinant sCD14 (Durieux et al). The sCD14 specific mouse monoclonal antibody , MY4 (IgG2b, Coulter, I.G., Zürich, Switzerland) was used for the detection of sCD14 in bovine milk. A rabbit polyclonal antibody specific for the 8 C-terminus peptide present in the β form of sCD14 was used for the analysis.

Milk samples were diluted (from 1:100 to 1:6) with Laemmli reducing sample buffer, boiled, loaded onto precasted 12.5 % polyacrylamide gels (Phastgels, Pharmacia AG, Switzerland) and electrophoresed in the presence of SDS in a PhastSystem® (pharmacia AG). Proteins were transferred to Hybond-ECL nitrocellulose membranes (Amersham) in transfer buffer (48 mM Tris, 39 mM glycerine, pH 9.0, 20% methanol) for 16 min at 30 mA/gel using a semi-dry transfer cell (Bio-Rad). SCD14 glycoproteins were detected by incubation of the blots with the antibodies and visualized by enhanced chemiluminescence (Amersham), following the manufacturer's instructions (lumininescence was detected by short exposure to Hyperfilm MP films).

Results demonstrate that the normal human plasma contain the typical 55 kDa β and 49 kDa α forms already well documented. The α polypeptide is the only sCD14 form detectable in all the breast milk samples tested how this is illustrated in figure 1A.

Consistent with this finding, Figure 1B, first two tracks from the left, show the lack of detection of the β peptide of sCD14 in a powdered bovine milk. For comparison, the lanes at the right side of the gel show the detection of sCD14 by the anti-α and anti-β polyclonal antibody in the breast milk sample (dilutions 1:100 and 1:50) and normal human plasma (1:50; PS: molecular weight markers).

Results also show the lack of detectable amounts of sCD14 (any form) in powdered bovine milk.

All human milk samples tested show a net predominance, if not an exclusive presence, of the α sCD14 form. Regarding the bovine milk preparations, the powdered milk showed no detectable amounts of any of the two sCD14 forms. It should be noted that fresh bovine milk did not show sCD14 either (data not shown).

Finally, bovine colostrom showed also a net predominance of an α sCD14 form (data not shown).

Test 3: sCD14 quantitative determinati n in breast milk

Concentration of sCD14 in human breast milk was determined by a human CD14-specific ELISA test (IBL, Germany). Samples taken in the first days of lactation had a higher amount of sCD14 than the overall average: 75.4± 19.1µg/ml compared to 52.9 ± 24.0µg/ml.

Samples taken after 9 days of lactation had a mean of 37.3 ±10.9 µg/ml that is below the average. Therefore the levels of sCD14 in milk appears to depend on the time elapsed after delevery

Test 4: Functional effect of milk-derived sCD14

In vitro stimulation studies of astrocytoma (U373) cell lines with several dose of lipopolysaccharides, 10pg/ml to 5pg/ml, in the presence and/or absence of human or bovine milk, human or foetal calf serum, and anti-CD14 antibodies were carried out.

The human astrocytoma cell line U373 (CD14 negative) was stimulated with varying concentrations of lipopolysaccharides, 1pg/ml to 10ng/ml, in the presence or absence of human AB serum (10% or 1%) and soluble recombinant human CD14 (3 ug/ml). After 48h culture at 37°C, culture supernatants were collected and tested for IL-6 release by ELISA . The levels of IL-6 were compared with those detected by stimulating the U373 with the same amounts of lipopolysaccharides in the absence of serum and the presence of 1% human milk. In some experiments, the anti-CD14 specific mAb MEM-18 and MY4 (15ug/ml), which binds to the same epitope as lipopolysaccharides on the sCD14 molecule, was used to block the stimulation mediated by the human milk or serum.

Neither lipopolysaccharides alone nor 0.5% or 1% human milk alone are able to induce significant levels of IL-6. However , lipopolysaccharides were capable of inducing substantial amounts of IL-6 in the presence of human milk (0.5 and 1%). This effect was abrogated by two CD14-specific monoclonal, antibodies , MEM-18 and MY4, but not by an irrelevant monolconal antibody (iMab MOPC-21). MEM-18 and MY4 were also able to block the release of IL-6 induced by lipopolysaccharides in the presence of 10 AB human serum, albeit partially, suggesting that other mechanisms of IL-6 release independent of CD14 may operate in human serum but not in milk.

In conclusion milk-derived sCD14 is biologically active and capable of mediating cell activation by lipopolysaccharides. Subtle differences in the biological activities are observed between serum sCD14 and milk-derived sCD14.

Test 5: Production of sCD14 from natural sources.

Purification of milk-derived sCD14 with monoclonal antibodies anti-sCD14, production of polyclonal antibody anti-milk-derived sCD14 and large scale purification immunoaffiniity chromatography and ion exchange chromatography .

a. Immunoaffinity chromatography for purification of sCD14 from milk (bovine, buffalo, goat or sheep)

Small scale purification of sCD14 from milk are performed by immunoaffinity chromatography using the purified monoclonal antibody anti-sCD14, MY4 (Coulter Immunotech, USA). Briefly, diluted milk sample is applied to an anti-CD14-Sepharose 4B matrix. After washing, the column is eluted with 100 mM Glycine.HCl, pH 2.5. Fractions are collected and neutralized. The sCD14 content of each fraction is determined by an anti-sCD14 ELISA test (IBL, Hamburg, Germany), and the purity is analysed by SDS-PAGE and silver staining. The selected fractions are pooled and kept in –20°C until further use.

Polyclonal antibodies against milk-derived sCD14 are obtained by immunisation of rabbits with the antigen-adjuvant mixture. Briefly, an emulsion composed of 0.5 mg/ml purified sCD14 and 2 ml complete Freund adjuvant (Sigma, St Louis, MO) with insoluble Mycobacterium tuberculosis bacilli is injected into multiple intamuscular sites. The animal bleed 14 days following the first immunisation. Booster immunisation using incomplete Freund adjuvant (Sigma) is performed 6 weeks after priming immunisation and with intervals of 2-3 weeks thereafter. Bleedings are always performed 10-14 days after immunisation. The antibody titers are performed by ELISA.

Small scale purification of sCD14 from milk are performed by immunoaffinity chromatography using the purified polyclonal antiserum.

Example 1

Formula for low-birth-weight infants, in powder form is prepared.

The formula has the composition (per 100 g of powder) which is described in the table I below.

Table I

Nutrient	Unit	Amount
Total fat	g	24
Total protein	g	14.4
Total carbohydrates	g	55.9
sCD14	mg	20
Sodium	mg	180
Potassium	mg	530
Chloride	mg	280
Calcium	mg	490
Phosphorus	mg	320
Magnesium	mg	54
Manganese	µg	34
Vitamin A	IU	1500
Vitamin D	IU	490
Vitamin E	IU	9.8
Vitamin K_1	µg	59
Vitamin C	mg	79
Vitamin B_1	mg	0.29
Vitamin B_2	mg	0.66
Niacin	mg	4.9
Vitamin B_6	mg	0.37
Folic acid	µg	290
Pantothenic acid	mg	2.2
Vitamin B_{12}	µg	1.1
Biotin	µg	11
Choline	mg	37

Nutrient	Unit	Amount
Inositol	mg	22
Taurine	mg	39
Carnitine	mg	7.9
Iron	mg	7.4
Iodine	µg	49
Copper	mg	0.44
Zinc	mg	3.7

The formula is reconstituted by mixing 142 g of powder to 900 mL of water to give 1 L of ready-to-drink preparation. The composition given above can vary to accommodate for local directives concerning the amounts of specific ingredients. Other trace elements (e.g, selenium, chromium, molybdenum, fluoride) may be added in adequate amount according to age. Nucleosides and/or nucleotides can also be present.

Example 2

Starter formula for infants (from birth to 4-5 months), in powder form is prepared.

The formula has the composition (per 100 g of powder) which is described in the table II below.

Table II

Nutrient	Unit	Amount
Total fat	g	25.8
Total protein	g	11.5
Total carbohydrates	g	57.8
sCD14	mg	20
Sodium	mg	120
Potassium	mg	460
Chloride	mg	360
Calcium	mg	320

Nutrient	Unit	Amount
Phosphorus	mg	160
Magnesium	mg	35
Manganese	µg	36
Vitamin A	IU	1500
Vitamin D	IU	310
Vitamin E	IU	6.1
Vitamin K_1	µg	42
Vitamin C	mg	41
Vitamin B_1	mg	0.31
Vitamin B_2	mg	0.69
Niacin	mg	3.8
Vitamin B_6	mg	0.38
Folic acid	µg	46
Pantothenic acid	mg	2.3
Vitamin B_{12}	µg	1.1
Biotin	µg	11
Choline	mg	38
Inositol	mg	23
Taurine	mg	41
Carnitine	mg	8.2
Iron	mg	6.1
Iodine	µg	25
Copper	mg	0.31
Zinc	mg	3.8

The formula is reconstituted by mixing 132 g of powder to 900 mL of water to give 1 L of ready-to-drink preparation. The composition given above can vary to accommodate for local directives concerning the amounts of specific ingredients. Other trace elements (e.g. selenium, chromium, molybdenum, fluoride) may be added in adequate amount according to age. Nucleosides and/or nucleotides can also be present.

Example 3

A formula for infants, from 5 months of age, is prepared.

The formula has the composition (per liter of ready to use preparation) which is described in the table III below.

Table III

Nutrient	Unit	Amount
Total fat	g	29.4
Total protein	g	22.4
Total carbohydrates	g	78.9
sCD14	mg	25
Sodium	mg	320
Potassium	mg	1060
Chloride	mg	760
Phosphorus	mg	680
Calcium	mg	820
Magnesium	mg	73
Manganese	µg	41
Vitamin A	IU	2700
Vitamin D	IU	600
Vitamin E	IU	8
Vitamin K_1	µg	30
Vitamin C	mg	67
Vitamin B_1	mg	1
Vitamin B_2	mg	1.6
Niacin	mg	18
Vitamin B_6	mg	1.3
Folic acid	µg	200
Pantothenic acid	mg	4.7
Vitamin B_{12}	µg	1.3
Biotin	µg	23
Choline	mg	67

Nutrient	Unit	Amount
Inositol	mg	34
Iron	mg	11
Iodine	µg	140
Copper	mg	0.8
Zinc	mg	8

The composition given above can vary to accommodate for local directives concerning the amounts of specific ingredients. Other trace elements (e.g. selenium, chromium, molybdenum, fluoride) may be added in adequate amount according to age. Nucleosides and/or nucleotides can also be present.

Example 4

A formula for infants, from 5 months of age, containing partly hydrolyzed protein with low allergenicity, in powder form is prepared. The formula has the composition (per 100 g of powder) which is described in the followed table IV.

Table IV

Nutrient	Unit	Amount
Total fat	g	22
Total protein	g	15.2
Total carbohydrates	g	57
sCD14	mg	20
Sodium	mg	170
Potassium	mg	580
Chloride	mg	340
Calcium	mg	540
Phosphorus	mg	230
Magnesium	mg	39
Manganese	µg	29
Vitamin A	IU	1900
Vitamin D	IU	440
Vitamin E	IU	5.8

Nutrient	Unit	Amount
Vitamin K_1	µg	22
Vitamin C	mg	49
Vitamin B_1	mg	0.73
Vitamin B_2	mg	1.2
Niacin	mg	13
Vitamin B_6	mg	0.97
Folic acid	µg	150
Pantothenic acid	mg	3,4
Vitamin B_{12}	µg	0.97
Biotin	µg	17
Choline	mg	49
Inositol	mg	24
Iron	mg	8.3
Iodine	µg	100
Copper	mg	0.58
Zinc	mg	5.8

The formula is reconstituted by mixing 138 g of powder to 900 mL of water to give 1 L of ready-to-drink preparation. The composition given above can vary to accommodate for local directives concerning the amounts of specific ingredients. Other trace elements (e.g. selenium, chromium, molybdenum, fluoride) may be added in adequate amount according to age. Nucleosides and/or nucleotides can also be present.

Example 5

A formula that can be administered to infant suffering from bovine milk allergy, comprising ultrafiltered/microfiltered extensively hydrolyzed protein, in powder form is prepared.
The formula has the composition (per 100 g of powder) which is described in the following table V.

Table V

Nutrient	Unit	Amount
Total fat	g	24
Total protein	g	16.5
Total carbohydrates	g	52
sCD14	mg	20
Sodium	mg	290
Potassium	mg	600
Chloride	mg	500
Calcium	mg	400
Phosphorus	mg	250
Magnesium	mg	60
Manganese	µg	337
Vitamin A	IU	1200
Vitamin D	IU	290
Vitamin E	IU	5.8
Vitamin K_1	µg	26
Vitamin C	mg	39
Vitamin B_1	mg	0.29
Vitamin B_2	mg	0.63
Niacin	mg	3.6
Vitamin B_6	mg	0.34
Folic acid	µg	43
Pantothenic acid	mg	2.2
Vitamin B_{12}	µg	0.96
Biotin	µg	11
Choline	mg	58
Inositol	mg	29
Taurine	mg	39
Carnitine	mg	14
Iron	mg	7.2
Iodine	µg	39
Copper	mg	0.39
Zinc	mg	3.4

Nutrient	Unit	Amount
Chromium	µg	14
Molybdenum	µg	39
Fluoride	µg	140

The formula is reconstituted by mixing 150 g of powder to 900 ml of water to give 1 l of ready-to-drink preparation. The composition given above can vary to accommodate for local directives concerning the amounts of specific ingredients. Other trace elements (e.g. selenium) may be added in adequate amount according to age. Nucleosides and/or nucleotides can also be present.

Claims

1. An enteral formula composition comprising an effective amount of sCD14 or any biologically active derivative thereof.

2. The use of sCD14 or any biologically active derivative thereof for the manufacture of a dietary composition for the treatment or prevention of disorders of the gastro-intestinal tract of mammals.

3. The use of sCD14 or any biologically active derivative thereof for the manufacture of a dietary composition for contributing to normal intestinal development during neonatal colonisation.

4. The use according to claim 2, for the treatnent or prevention of disorders associated to prematurity and low birth weight such as necrotising entero-colitis.

5. An enteral formula composition or use according to any of claims 1 to 4, wherein the sCD14 is milk-derived sCD14α form or any biologically active derivative thereof.

6. An enteral formula composition or use according to any of claims 1 to 5, wherein at least 25 µg/ml of SCD14 or any biologically active derivative thereof is present.

7. An enteral formula composition or use according to any of claims 1 to 6, wherein the composition comprises 1.8 – 4.5 g of protein / 100 kcal.

8. An enteral formula composition or use according to any of claims 1 to 7 which contains carbohydrate and fat in a weight ratio of 60:40.

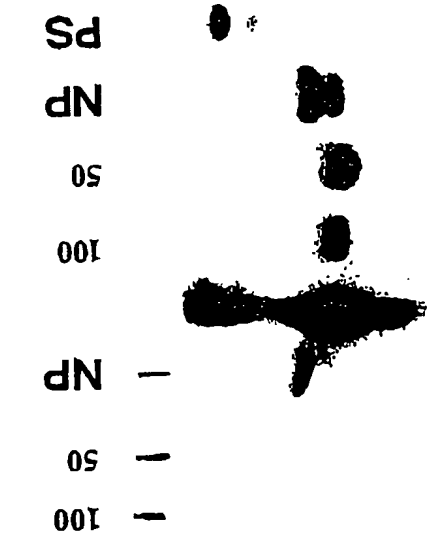
9. An enteral formula composition or use according to any of claims 1 to 8 which further compises AMP, GMP, CMP, and/or UMP.

10. A process to manufacture an enteral formula comprising the step of adding sCD14α or any biologically active derivative thereof, to the formula.

Abstract

Enteral Composition

Enteral composition comprising an effective amount of sCD14 or any biologically active derivative thereof.



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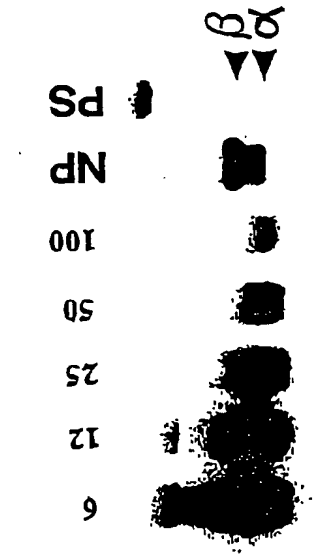

1A

FIG 1/1